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(57) Abstract

A composition suitable for incorporation into foods, beverages, pharmaceuticals, nutraceuticals and the like comprises one or more phytosterols, phytostanols or mixtures of both, treated to enhance the solubility and dispersability thereof.

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COMPOSITIONS COMPRISING PHYTOSTEROL AND/OR PHYTOSTANOL HAVING ENHANCED SOLUBILITY AND DISPERSABILITY

FIELD OF THE INVENTION

This present invention relates to the field of phytosterol-based compositions suitable for incorporation into foods, pharmaceuticals, nutraceuticals and the like and to methods of making the same.

BACKGROUND OF THE INVENTION

While recent advances in science and technology are helping to improve quality and add years to human life, the prevention of atherosclerosis, the underlying cause of cardiovascular disease ("CVD") has not been sufficiently addressed. Research to date suggest that cholesterol may play a role in atherosclerosis by forming atherosclerotic plaques in blood vessels, ultimately cutting off blood supply to the heart muscle or alternatively to the brain or limbs, depending on the location of the plaque in the arterial tree (1,2). Overviews have indicated that a 1% reduction in a person's total serum cholesterol yields a 2% reduction in risk of a coronary artery event (3). Statistically, a 10% decrease in average serum cholesterol (e.g. from 6.0 mmol/L to 5.3 mmol/L) may result in the prevention of 100,000 deaths in the United States annually (4).

Sterols are naturally occurring triterpenoids that perform many critical cellular functions. Phytosterols such as campesterol, stigmasterol and beta-sitosterol in plants, ergosterol in fungi and cholesterol in animals are each primary components of cellular and sub-cellular membranes in their respective cell types. The dietary source of phytosterols in

humans comes from plant materials i.e. vegetables and plant oils. The estimated daily phytosterol content in the conventional western-type diet is approximately 60-80 milligrams in contrast to a vegetarian diet which would provide about 500 milligrams per day.

Phytosterols have received a great deal of attention due to their ability to decrease serum cholesterol levels when fed to a number of mammalian species, including humans. While the precise mechanism of action remains largely unknown, the relationship between cholesterol and phytosterols is apparently due in part to the similarities between the respective chemical structures (the differences occurring in the side chains of the molecules). It is assumed that phytosterols displace cholesterol from the micellar phase and thereby reduce its absorption.

Over forty years ago, Eli Lilly marketed a sterol preparation from tall oil and later from soybean oil called Cytellin™ which was found to lower serum cholesterol by about 9% according to one report (5). Various subsequent researchers have explored the effects of sitosterol preparations on plasma lipid and lipoprotein concentrations (6) and the effects of sitosterol and campesterol from soybean and tall oil sources on serum cholesterols (7). A composition of phytosterols which has been found to be highly effective in lowering serum cholesterol is disclosed in PCT/CA95/00555 and comprises no more than 70% by weight beta-sitosterol, at least 10% by weight campesterol and stigmastanol. It is hypothesized in this patent application (which has already issued to patent in some countries) that there may be some form of synergy between the constituent phytosterols.

Given that phytosterols in various combinations have been proven to have wide clinical and dietary applications in lowering total and low density lipoprotein cholesterol, the key problem now facing researchers in this field is the adaptation of the phytosterol delivery system. Studies have investigated how the form (for example, crystalline, suspension, granular) in which the phytosterols are dosed impacts on their ability to lower serum cholesterol levels. Phytosterols are highly hydrophobic, do not dissolve to any significant degree in the micellar phase in the digestive tract and therefore are not

capable of efficiently blocking cholesterol absorption. Oils and fats are capable to a limited but not satisfactory degree of dissolving free phytosterols. Since only solubilized phytosterols inhibit the absorption of cholesterol, this "delivery" problem must be adequately addressed.

Early research focused on grinding or milling the phytosterols in order to enhance their solubility (US Patent Serial Nos: 3,881,005 and 4,195,084 both to Eli Lilly). In addition, researchers have looked to the esterification of phytosterols in order to enhance their solubility in delivery systems. German Patent 2035069/January 28, 1971 (analogous to US Patent Serial No. 3,751,569) describes the addition of phytosterol fatty acid esters to cooking oil. The esterification is carried out between a free sterol and a fatty acid anhydride, with perchloric acid as the catalyst. The significant drawback to this process, along with others, is the use of non-food grade catalysts and reagents.

US Patent Serial No. 4,588,717 to David E. Mitchell Medical Research Institute describes a vitamin supplement which comprises a fatty acid ester of a phytosterol, wherein the fatty acid forming the ester has from about 18 to 20 carbon atoms in the main carbon chain.

US Patent No. 5,270,041 to Marigen S.A. teaches the use of small amounts of sterols, their fatty acid esters and glucosides for the treatment of tumours. The method of preparation of these compositions involving the use of hazardous chemical reagents effectively precludes their use in foods or as dietary additives.

Other research has demonstrated that phytostanols, the 5 alpha saturated derivatives of phytosterols, are more effective as therapeutic agents in lowering serum cholesterol on a molecular weight basis than phytosterols (8). Similarly, in a further comparison, sitosterols infused into the GI tract resulted in a 50% reduction in serum cholesterol as opposed to an 85% reduction when sitostanols were infused (9). The advantages of stanols over sterols with respect to inhibition of cholesterol absorption from the GI tract are two-fold. Firstly, stanols are more chemically stable than their unsaturated counterparts in heat and air due to the absence of carbon-carbon bonds in the former.

Secondly, stanols are more effective at lowering serum cholesterol on a molecular weight basis than their unsaturated counterparts.

US Patent Serial No. 5,502,045 to Raision Tehtaat Oy AB (hereinafter the "Raision Patent") describes the preparation of a beta-sitostanol fatty acid ester mixture prepared by interesterifying beta-sitostanol with a fatty acid ester containing from 2 to 22 carbon atoms in the presence of an interesterification catalyst. This process renders the sitostanol appreciably more soluble in fats and oils.

South African Patent Application 967616 also to Raision Tehtaat Oy AB (hereinafter the "SA Raision Patent") describes a similar composition to that in the Raision Patent but which further contains at least 10% campestanol obtained by hydrogenation of the phytosterol mixture.

US Patent No.5,244,887 to Straub discloses a method of making a food additive composition which comprises dissolving a stanol (sitostanol; clionastanol; 22,23-dihydrobrassicastanol; campestanol and mixtures thereof) with an edible solubilizing agent, an anti-oxidant and a carrier or dispersant.

Although the Raision Patent and the Raision SA Patent both attempt to produce a phytostanol delivery system which is stable and effective, there are significant problems with the long-term stability of these esterified products due to the ultimate oxidation of the unsaturated fatty acid moiety.

It is an object of the present invention to obviate or mitigate the above disadvantages.

SUMMARY OF THE INVENTION

The present invention provides a composition suitable for use alone or for incorporation into foods, beverages, pharmaceuticals, nutraceuticals and the like which comprises one or more phytosterols, phytostanols or mixtures of both treated to enhance the solubility and dispersability thereof.

The present invention further comprises foods, beverages, pharmaceuticals, nutraceuticals and the like which comprise a composition of one or more phytosterols, phytostanols or mixtures of both treated to enhance the solubility and dispersability thereof. These "formulations" include, but are not limited to, the treated composition incorporated into edible oils and fat-based foods (such as margarines, butter, mayonnaise, dressing, shortenings, and cheeses), and formed into suspensions, emulsions, microemulsions, liposomes, niosomes and general hydrated lipid phases. The composition additionally may be incorporated into numerous pharmaceutical dosage forms as described in detail below.

The present invention further comprises the use of a composition which comprises one or more phytosterols, phytostanols or mixtures of both, treated to enhance the solubility and dispersability thereof, to lower serum cholesterol in animals, including humans.

The present invention further comprises methods of making a composition suitable for incorporation into foods, beverages, pharmaceuticals, nutraceuticals and the like which comprises enhancing the solubility and dispersability of one or more phytosterols, phytostanols or mixtures of both by any of the techniques described in more detail hereinbelow.

The composition of the present invention, which comprises one or more phytosterols, phytostanols or mixtures of both treated to enhance the solubility and dispersability thereof, has marked advantages over the phytosterol/stanol compositions previously known and described in the art. The composition of the present invention is more soluble and dispersable in both lipid-based and aqueous systems which is critically important due to the fact that only solubilized phytosterols and phytostanols inhibit the absorption of cholesterol in the digestive tract. Furthermore, the techniques described herein to enhance the solubility and dispersability of phytosterol compositions and phytostanol compositions (or mixtures thereof) facilitate the incorporation of these compositions into foods, beverages, nutraceuticals and pharmaceuticals.

PREFERRED EMBODIMENTS OF THE INVENTION

According to one aspect of the present invention, there is provided a composition suitable for incorporation into foods, beverages, pharmaceuticals, nutraceuticals and the like which comprises one or more phytosterols, phytostanols or mixtures of both treated to enhance the solubility and dispersability thereof.

As used herein, the term "phytosterol" includes all phytosterols without limitation, for example: sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized forms and derivatives thereof, including isomers. The term "phytostanol" includes all saturated or hydrogenated phytosterols and all natural or synthesized forms and derivatives thereof, including isomers. It is to be understood that modifications to the phytosterols and phytostanols i.e. to include side chains also falls within the purview of this invention. It is also to be understood that this invention is not limited to any particular combination of phytosterols and/or phytostanols forming a composition. In other words, any phytosterol or phytostanol alone or in combination with other phytosterols and phytostanols in varying ratios as required depending on the nature of the ultimate formulation may be treated to enhance the solubility and dispersability as described in the present invention. For example, the composition described in PCT/CA95/00555 which comprises no more than 70% by weight beta-sitosterol, at least 10% by weight campesterol and stigmastanol may be treated by the techniques of the present invention to yield a stable and favourably soluble product for incorporation into foods, beverages, pharmaceuticals and the the like.

The phytosterols for use in this invention may be procured from a variety of natural sources. For example, they may be obtained from the processing of plant oils (including aquatic plants) such as corn oil and other vegetable oils), wheat germ oil, soy extract, rice extract, rice bran, rapeseed oil, sesame oil and fish oil. Without limiting the generality of the foregoing, it is to be understood that there are other sources of phytosterols such as marine animals from which the composition of the present invention may be prepared. US Patent Serial No. 4,420,427 teaches the preparation of sterols from vegetable oil sludge using solvents such as methanol. Alternatively,

phytosterols may be obtained from tall oil pitch or soap, by-products of the forestry practise as described in PCT/CA95/00555, incorporated herein by reference.

There are numerous techniques of enhancing the solubility and dispersability of phytosterols and/or phytostanols which may successfully be used within the scope of the present invention. These techniques improve the effectiveness of the phytosterols and/or phytostanols in lowering serum cholesterol and ensure even distribution of the phytosterols and/or phytostanols throughout the food, beverage, pharmaceutical nutraceutical and the like to which they are added. Such enhancement may be achieved by a number of suitable mans such as, for eample, solubilizing or dispersing the phytosterol or phytostanol composition (or mixture thereof) to form emulsions, solutions and dispersions or self-emulsifying systems; reducing the particle size by mechanical grinding (milling, micronisation etc...), lyophilizing, spray drying, controlled precipitating or a combination thereof; forming solid dispersions, suspensions, hydrated lipid systems; forming inclusion complexations with cyclodextrins; and forming hydrotopes and formulations with bile salts and their derivatives.

Prior to the solubility/dispersability enhancement techniques of the present invention, it is preferred that the phytosterols and/or phytostanols be isolated from the source and formed into a solid powder through precipitation, filtration and drying, spray drying, lyophilization or by other conventional work-up techniques. This powder form may then be physically modified as described below to enhance the solubility and dispersability of the phytosterol and/or phytostanol in the chosen delivery medium.

Each of the tecnniques which may be used in accordance with the present invention are described below.

Emulsions

Emulsions are finely divided or colloidal dispersions comprising two immiscible phases, e.g. oil and water, one of which (the internal or discontinuous phase) is dispersed as droplets within the other (external or discontinuous phase). Thus an oil-in-water

emulsion consists of oil as the internal phase, dispersed water as the external phase, the water-in-oil emulsion being the opposite.

A wide variety of emulsified systems may be formed which comprise the composition of the present invention including standard emulsions, microemulsions and those which are self-emulsifying (emulsify on exposure to agitated aqueous fluids such as gastric or intestinal fluids).

Generally, emulsions may include oil and water phases, emulsifiers, emulsion stabilizers and optionally preservatives, flavouring agents, pH adjusters and buffers, chelating agents, antifoam agents, tonicity adjusters and anti-oxidants. Suitable emulsifiers (wherein bracketed numerals refer to the preferred HLB values) include: anionic surfactants such as alcohol ether sulfates, alkyl sulfates (30-40), soaps (12-20) and sulfosuccinates; cationic surfactants such as quaternary ammonium compounds; zwitterionic surfactants such as alkyl betaine derivatives; amphoteric surfactants such as fatty amine sulfates, difatty alkyl triethanolamine derivatives (16-17); and nonionic surfactants such as the polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, saturated fatty acids and alkyphenols, water-soluble polyethyleneoxy adducts onto polypropylene glycol and alkyl polypropylene glycol, nonylphenol polyethoxyethanols, castor oil polyglycol ethers, polypropylene/polyethylene oxide adducts, tributylphenoxy-polyethoxyethanol, polyethylene glycol, octylphenoxy-polyethoxyethanol, lanolin alcohols, polyoxyethylated (POE) alkyl phenols, POE fatty amides, POE fatty alcohol ethers, POE fatty amines, POE fatty esters, poloxamers (7-19), POE glycol monoethers (13-16), polysorbates and sorbitan esters. This list is not intended to be exhaustive as other emulsifiers are equally suitable.

Appropriate emulsion stabilizers include, but are not limited to, lyophilic colloids such as polysaccharides (e.g. acacia, agar, alginic acid, carrageenin, guar gum, karaya gum, tragacanth xanthan gum), amphoterics (e.g. gelatin) and synthetic or semi-synthetic polymers (e.g. carbomer resins, cellulose ethers, carboxymethyl chitin, polyethylene glycol-n (ethylene oxide polymer H(OCH2CH2)nOH); finely divided solids including clays (e.g. attapulgite, bentonite, hectorite, kaolin, magnesium aluminum silicate and montmorillonite), microcrystalline cellulose oxides and hydroxides (e.g. aluminum

hydroxide. magnesium hydroxide and silica); and cybotactic promoters/gellants including amino acids, peptides, proteins lecithin and other phospholipids and poloxamers.

Suitable anti-oxidants for use in the formation of emulsions include: chelating agents such as citric acid, EDTA, phenylalanine, phosphoric acid, tartaric acid and tryptophane; preferentially oxidized compounds such as ascorbic acid, sodium bisulfite and sodium sulfite; water soluble chain terminators such as thiols and lipid soluble chain terminators such as alkly gallates, ascorbyl palmitate, t-butyl hydroquinone, butylated hydroxyanisole, butylated hydroxytoluene, hydroquinone, nordihydroguaiaretic acid and alpha-tocopherol. Suitable preservatives, pH adjustment agents, and buffers, chelating agents, osmotic agents, colours and flavouring agents are discussed hereinbelow under "Supensions", but are equally applicable with respect to the formation of emulsions.

The general preparation of emulsions is as follows: the two phases (oil and water) are separately heated to an appropriate temperature (the same in both cases, generally 5-10°C above the melting point of the highest melting ingredients in the case of a solid or semi-solid oil, or where the oil phase is liquid, a suitable temperature as determined by routine experimentation). Water-soluble components are dissolved in the aqueous (water) phase and oil-soluble components are dissolved in the oil phase. To create an oil-in water emulsion, the oil phase is vigorously mixed into the aqueous phase to create a suitable dispersion and the product is allowed to cool at a controlled rate with stirring. A water-in-oil emulsion is formed in the opposite fashion i.e. the water phase is added to the oil phase. When hydrophillic colloids are a part of the system as emulsion stabilizers, a phase inversion technique may be employed whereby the colloid is mixed into the oil phase rather than the aqueous phase, prior to addition to the aqueous phase. In using the oil-based composition of the present invention, which is semi-solid, it is preferred to add the composition to the oil phase prior to heating.

Microemulsions, characterized by a particle size at least an order of magnitude smaller (10-100 nm) than standard emulsions and defined as "a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid"

(14), may also be formed comprising the composition of the present invention. In a preferred form, the microemulsion comprises a surfactant or surfactant mixture, a co-surfactant,(usually a short chain alcohol) the oil-based composition of the present invention, water and optionally other additives.

This system has several advantages as a delivery system for the phytosterols or phytostanols or mixtures thereof having relatively high lipophilicity. Firstly, microemulsions tend to be created spontaneously, that is, without the degree of vigorous mixing required to form standard emulsions. From a commercial perspective, this simplifies the manufacturing process. Secondly, microemulsions may be sterilized using microfiltration techniques without breaking the microstructure due to the small diameter of the microdroplets. Thirdly, microemulsions are highly thermodynamically stable. Fourthly, microemulsions possess high solubilizing power which is particularly important as they allow for an increased solubilization of the poorly hydrosoluble phytosterols and phytostanols.

Surfactant or surfactant mixtures which are suitable for use in the formation of microemulsions can be anionic, cationic, amphoteric or non-ionic and possess HLB (hydrophile-lipophile balance) values within the range of 1-20, more preferably in the ranges 2-6 and 8-17. Especially preferred agents are non-ionic surfactants, selected from the group consisting of polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, saturated fatty acids and alkyphenols, water-soluble polyethyleneoxy adducts onto polypropylene glycol and alky polypropylene glycol, nonylphenol polyethoxyethanols, castor oil polyglycol ethers, polypropylene/polyethylene oxide adducts, tributylphenoxy-polyethoxyethanol, polyethylene glycol, octylphenoxy-polyethoxyethanol, lanolin alcohols, polyoxyethylated (POE) alkyl phenols, POE fatty amides, POE fatty alcohol ethers, POE fatty amines, POE fatty esters, poloxamers (7-19), POE glycol monoethers (13-16), polysorbates and sorbitan esters.

There are many methods known and used by those skilled in the art for making microemulsions. In a preferred method of forming microemulsions of the present invention, a surfactant, a co-surfactant and the phytosterol, phytostanols or mixtures

thereof (pre-dissolved in a suitable proportion of an appropriate oil) is mixed and then titrated with water until a system of desired transparency is obtained.

In a further preferred embodiment, the formation of microemulsions may be achieved by mixing the phytosterols or phytostanols or mixtures thereof with hydrotropic agents and food-grade surfactants (refer to 11).

Solutions and Dispersions

Phytosterols or phytostanols or mixtures thereof may be dissolved or dispersed in a suitable oil vehicle and used in this form, for example, in general food usage, in basting meats and fish, and for incorporation into animal feeds.

Suitable solubilizing agents include all food grade oils such as plant oils, marine oils such as fish oil and vegetable oils, monoglycerides, diglycerides, triglycerides, tocopherols and the like, and mixtures thereof.

Self-Emulsifying Systems

Phytosterols or phytostanols or mixtures thereof may be mixed with appropriate excipients, for example, surfactants, emulsion stabilizers (described above) and the like, heated (if necessary) and cooled to form a semi-solid product capable of forming a spontaneous emulsion on mixing with water. This semi-solid product may be used in numerous other forms such as filler material in two-piece hard or soft gelatin capsules, or may be adapted for use in other delivery systems.

Reducing Particle Size

Many techniques of particle size reduction are suitable for use within the present invention including, *inter alia*, dry milling, micropulverization, fluid energy grinding, controlled precipitation, lyophilisation and spray-drying. Each of these techniques is well known in the art and will not be discussed in any detail other than to provide reference to 12 and 13, the former showing preferred processes of spray-drying and the latter summarizing the other techniques listed above.

It has been found that reducing the particle size to under 500um and most preferably under 20um allows suitable dispersability/solubility of the composition in the carriers and dosage forms described further below.

Solid Dispersions

An alternative means of increasing the solubility/dispersability of phytosterols, phytostanols or mixtures thereof involves the use of solid dispersion systems. These dispersions may include molecular solutions (eutectics), physical dispersions or a combination of both.

For example, solid dispersions may typically be prepared by utilizing water-soluble polymers as carriers. Without limitation, these carriers may include, either alone or in combination: solid grade polyethylene glycols (PEG's), with or without the addition of liquid grade PEG's; polyvinylpyrrolidones or their co-polymers with vinyl acetate and cellulose ethers and esters. Other excipients, such as additional members of the glycol family e.g. propylene glycol, polyols,e.g. glycerol etc.. may also be included in the dispersions.

Solid dispersions may be prepared by a number of ways which are familiar to those in the art. These include, without limitation, the following methods:

(a) fusing the ingredients, followed by controlled cooling to allow solidification and subsequent mechanical grinding to produce a suitable powder. Alternatively, the molten (fused) dispersion may be sprayed into a stream of cooled air in a spray drier to form solid particles (prilling) or passed through an extruder and spheroniser to form solid masses of a controlled particle size. In a further alternative, the molten dispersion is filled directly into two-piece hard gelating capsules;

(b) dissolving the ingredients in a suitable solvent system (organic, mixed organic, organic-aqueous) and then removing the solvents e.g. by evaporating at atmospheric pressure or in vacuo, spray drying, lyophilizing and the like; or, in a variation of the foregoing, and

(c) dissolving the ingredients in a suitable solvent system, subsequently precipitating them from solution by the use of an immiscible solvent in which the ingredients have little or no solubility, filtration, removing the solvent, drying and optionally grinding to provide a suitable powder form.

Other commercially available agents for enhancing solubility of the phytosterols or phytostanols or mixtures thereof through the formation of solid dispersions are considered to fall within the purview of this invention. For example, the commercial excipient marketed under the trade-mark Gelucire™ by Gattefosse comprising saturated polyglycolised glycerides may readily be used herein.

Suspensions

Suspensions, which may be used to enhance the solubility and/or dispersability of the phytosterols, phytostanols or mixtures thereof, comprise a solid, perhaps finely divided, internal phase dispersed in an oily or aqueous external phase (the vehicle). In addition, the solid internal phase may be added to an emulsion as described above during its' formation to produce a delivery system having properties common to both suspensions and emulsions.

Numerous excipients, which are commonly used in the art, may be suitable for producing a suspension within the scope of the present invention. Typically, a suspension comprises an oily or aqueous vehicle, the dispersed (suspended) internal phase, dispersing and/or wetting agents (surfactants), pH adjustment agents/buffers, chelating agents, antioxidants, agents to adjust ionic strength (osmotic agents) colours, flavours, substances to stabilize the suspension and increase viscosity (suspending agents) and preservatives.

Appropriate vehicles include, but are not limited to: water, oils, alcohols, polyols, other edible or food grade compounds in which the phytosterol composition is partially or not soluble and mixtures thereof. Appropriate dispersing agents include, but are not limited to: lecithin; phospholipids; nonionic surfactants such as polysorbate 65, octoxynol-9, nonoxynol-10, polysorbate 60, polysorbate 80, polysorbate 40, poloxamer 235,

polysorbate 20 and poloxamer 188; anionic surfactants such as sodium lauryl sulfate and docusate sodium; fatty acids, salts of fatty acids, other fatty acid esters, and mixtures thereof.

Agents/buffers for pH adjustment include citric acid and its salts, tartaric acid and its salts, phosphoric acid and its salts, acetic acid and its salts, hydrochloric acid, sodium hydroxide and sodium bicarbonate. Suitable chelating agents include edetates (disodium, calcium disodium and the like), citric acid and tartaric acid. Suitable antioxidants include ascorbic acid and its salts, ascorbyl palmitate, tocopherols (especially alpha-tocopherol), butylated hydroxytoluene, butylated hydroxyanisole, sodium bisulfite and metabisulfite. Suitable osmotic agents include monovalent, divalent and trivalent electrolytes, monosaccharides and disaccharides. Suitable preservatives include parabens (Me, Et, Pr, Bu), sorbic acid, thimerosal, quaternary ammonium salts, benzyl alcohol, benzoic acid, chorhexidine gluconate and phenylethanol. Colours and flavours may be added as desired and may be selected from all nature, natural-identical and synthetic varieties.

Hydrated Lipid Systems

In a further embodiment of the present invention, the solubility/dispersability of phytosterols, phytostanols or mixtures thereof may be enhanced by the formation of phospholipid systems such as liposomes and other hydrated lipid phases, by physical inclusion. This inclusion refers to the entrapment of molecules without forming a covalent bond and is widely used to improve the solubility and subsequent dissolution of active ingredients.

Hydrated lipid systems, including liposomes, can be prepared using a variety of lipid and lipid mixtures, including phospholipids such as phosphatidylcholine (lecithin), phosphodiglyceride and sphingolipids, glycolipids, cholesterol and the like. The lipids may preferably be used in combination with a charge bearing substances such as charge-bearing phospholipids, fatty acids, and potassium and sodium salts thereof in order to stabilize the resultant lipid systems. A typical process of forming liposomes is as follows:

1) dispersion of lipid or lipids and the phytosterols or phytostanols or mixtures thereof in an organic solvent (such as chloroform, dichloromethane, ether, ethanol or other alcohol, or a combination thereof). A charged species may be added to reduce subsequent aggregation during liposome formation. Antioxidants (such as ascorbyl palmitate, alpha-tocopherol, butylated hydroxytoluene and butylated hydroxyanisole) may also be added to protect any unsaturated lipids, if present;
2) filtration of the mixture to remove minor insoluble components;
3) removal of solvents under conditions (pressure, temperature) to ensure no phase separation of the components occur;
4) hydration of the "dry" lipid mixture by exposure to an aqueous medium containing dissolved solutes, including buffer salts, chelating agents, cryoprotectorants and the like; and
5) reduction of liposome particle size and modification of the state of lamellarity by means of suitable techniques such as homogenization, extrusion etc..

Any procedure for generating and loading hydrated lipid with active ingredients, known to those skilled in the art, may be employed within the scope of this invention. For example, suitable processes for the preparation of liposomes are described in references 14 and 15, both of which are incorporated herein by reference. Variations on these processes are described in US Patent Serial No. 5,096,629 which is also incorporated herein by reference.

US Patent Serial No. 4,508,703 (also incorporated herein by reference) describes a method of preparing liposomes by dissolving the amphiphillic lipidic constituent and the hydrophobic constituent to form a solution and thereafter atomizing the solution in a flow of gas to produce a pulverent mixture.

Cyclodextrin Complexes

Cyclodextrins are a class of cyclic oligosaccharide molecules comprising glucopyranose sub-units and having a toroidal cylindrical spatial configuration. Commonly available members of this group comprise molecules containing six (alpha-cyclodextrin), seven

(beta-cyclodextrin) and eight (gamma-cyclodextrin) glucopyranose molecules, with the polar (hydrophilic) hydroxyl groups oriented to the outside of the structure and the apolar (lipophilic) skeletal carbons and ethereal oxygens lining the interior cavity of the toroid. This cavity is capable of accomodating (hosting) the lipophilic moiety of an active ingredient (the guest molecule, here the phytosterol or phytostanol or mixture of both) by bonding in a non-covalent manner to form an inclusion complex.

The external hydroxyl substituents of the cyclodextrin molecule may be modified to form derivatives having improved solubility in aqueous media along with other desired enhancements, such as lowered toxicity, etc.. Examples of such derivatives are: alkylated derivatives such as 2,6-dimethyl-beta-cclodextrin; hydroxyalkylated derivatives such as hydroxypropyl-beta-cyclodextrin; branched derivatives such as diglucosly-beta-cyclodextrin; sulfoalkyl derivatives such as sulfobutylether-beta-cyclodextrin; and carboxymethylated derivatives such as carboxymethyl-beta-cyclodextrin. Other types of chemical modifications, known to those in the art, are also included within the scope of this invention.

The cyclodextrin complex often confers properties of improved solubility, dispersability, stability (chemical, physical and microbiological), bioavailability and decreased toxicity on the guest molecule (here, the phytosterols or phytostanols or mixtures thereof).

There are a number of ways known in the art to produce a cyclodextrin complex. Complexes may be produced, for example, by using the following basic methods: stirring the phytotsterol, phytostanol or mixture thereof into an aqueous or mixed aqueous-organic solution of the cyclodextrin, with or without heating; kneading, slurrying or mixing the cyclodextrin and the phytotsterol, phytostanol or mixture thereof in a suitable device with the addition of an appropriate quantity of aqueous, organic or mixed aqueous-organic liquid, with or without heating; or by physical admixture the cylcodextrin and the phytotsterol, phytostanol or mixture thereof using a suitable mixing device. Isolation of the inclusion complex so formed may be achieved by co-precipitation, filtration and drying; extrusion/spheronisation and drying; subdivision of the moist mass and drying; spray drying; lyophilization or by other suitable techniques

depending on the process used to form the cyclodextrin complex. A further optional step of mechanically grinding the isolated solid complex may be employed.

These cyclodextrin/phytosterol complexes enhance the solubility and dissolution rate and increase the stability of the phytosterols or phytostanols or mixtures thereof. For a review of cyclodextrin complexation, please refer to 16.

Complexation with Bile Salts

Bile acids, their salts and conjugated derivatives, suitably formulated, may be used to solubilize phytosterols, phytostanols or mixtures thereof, thereby improving the solubility and dispersion characteristics of these compositions. Examples of suitable bile acids include: cholic acid, chenodeoxycholic acid, deoxycholic acid, dehydrocholic acid, and lithocholic acid. Examples of suitable bile salts include: sodium cholate, sodium deoxycholate and their other salt forms. Examples of suitable conjugated bile acids include: glycochenodeoxycholic acid, glycholic acid, taurochenodeoxycholic acid, taurocholic acid, taurodeoxycholic acid and their salts.

A suitable system for solubilizing phytosterols or phytostanols or mixtures thereof consists of the sterol or stanol component plus one or more bile acids, salts or conjugated bile acids. Further materials may be added to produce formulations having additional solubilization capacity. These materials include, but are not limited to: phospholipids, glycolipids and monoglycerides. These ingredients may be formulated either in the solid phase or by the use of suitable solvents or carrier vehicles, with appropriate isolation and, optionally, particle size reduction using techniques described hereinabove.

Since bile acids and their derivatives have an unpleasant taste and may be irritating to the mucous membranes of the stomach and upper regions of the gastro-intestinal tract, a suitable enteric coating may be applied to the solid formulation particulates, using techniques known to those skilled in the art. Typical enteric coatings include, inter alia: cellulose acetate phthalate, cellulose acetate trimellitiate, hydroxypropImethylcellulose phthalate, hydroxypropImethylcellulose acetate succinate, poly (vinylaceate phthalate),

acrylate polymers and their derivatives (e.g. appropriate members of the Eudragit series), ethylcellulose or combinations thereof. Additional excipients may be added to the coating formulation to modify membrane functionality or to aid in the coating process (e.g. surfactants, plasticisers, channeling agents, permeability modifiers and the like). Coating formulation vehicles may comprise aqueous or organic systems, or mixtures of both.

Hydrotopic Complexation

Compounds which are capable of opening up the water structure associated with hydrophobic (lipophilic) and other molecules are referred to as hydrotopes. These compounds may be used to enhance the aqueous solubility of poorly water-soluble substances such as phytosterols, phytostanols and their esters. Examples of hydrotopes include, inter alia, sodium benzoate, sodium hydroxybenzoates, sodium salicylate, nicotinamide, sodium nicotinate, sodium gentisate, gentisic acid ethanolamide, sodium toluates, sodium aminobenzoates, sodium anthranilate, sodium butylmonoglycolsulfate, resorcinol and the like.

Complex formation, which is non-covalent in nature, may be achieved by mixing appropriate ratios of the phytosterols or phytostanols or mixtures thereof and the hydrotope or mixtures thereof in a suitable liquid vehicle, which may be aqueous, organic or a combination of both. Additional excipients such as surfactants, polyol, disaccharides etc.. may be added to facilitate complexation or to aid in dispersability. The resultant complex is isolated as a dry powder by any process known in the art (co-precipitation and drying, evaporation of the liquid vehicle, spray drying, lyophilization etc..). Particle size may be reduced by any standard technique such as those described previously herein, if desired. The resultant hydrotope complex may be used without further modification or may be compounded into a variety of other formulations or vehicles as required.

Methods of Use:

Any phytosterol or phytostanol or mixture thereof, treated as described herein to form a composition of enhanced solubility/dispersability, may be used as an effective agent to

lower serum cholesterol in animals, particularly humans. It is to be understood, however, that this composition is equally suited for administration to other animals, for example, in the form of veterinary medicines and animal foods. There are numerous modes or "vehicles" of delivery of this composition, accordingly, this invention is not intended to be limited to the following delivery examples.

1) Pharmaceutical Dosage Forms:

It is contemplated within the scope of the present invention that the composition of the present invention may be incorporated into various conventional pharmaceutical preparations and dosage forms such as tablets (plain and coated) for use orally, bucally or lingually, capsules (hard and soft, gelatin, with or without additional coatings) powders, granules (including effervescent granules), pellets, microparticulates, solutions (such as micellar, syrups, elixirs and drops), lozenges, pastilles, ampuls, emulsions, microemulsions, ointments, creams, suppositories, gels, and transdermal patches, modified release dosage forms together with customary excipients and/or diluents and stabilizers.

The composition of the present invention, adapted into the appropriate dosage form as described above may be administered to animals, including humans, orally, by injection (intra-venously, subcutaneously, intra-peritoneally, intra-dermally or intra-muscularly), topically or in other ways. Although the precise mechanism of action is unclear, the composition of the present invention, administered intra-venously, lowers serum cholesterol. It is believed that the phytosterol composition may have, in addition to the role as an inhibitor of cholesterol absorption in the intestine, a systemic effect on cholesterol homeostasis through bile acid synthesis, enterocycte and biliary cholesterol excretion, bile acid excretion and changes in enzyme kinetics and cholesterol transport between various compartments within the body (PCT/CA97/00474 which was published on January 15, 1998). See also paper to Peter Jones (under publication).

2) Foods/Beverages/Nutraceuticals:

In another form of the present invention, the composition of the present invention may be incorporated into foods, beverages and nutraceuticals, including, without limitation, the following:

1) Dairy Products –such as cheeses, butter, milk and other dairy beverages, spreads and dairy mixes, ice cream and yoghurt;
2) Fat-Based Products–such as margarines, spreads, mayonnaise, shortenings, cooking and frying oils and dressings;

3) Cereal-Based Products–comprising grains (for example, bread and pastas) whether these goods are cooked, baked or otherwise processed;

4) Confectionaries–such as chocolate. candies, chewing gum, desserts, non-dairy toppings (for example Cool Whip™), sorbets, icings and other fillings;

5) Beverages– whether alcoholic or non-alcoholic and including colas and other soft drinks, juices, dietary supplement and meal replacement drinks such as those sold under the trade-marks Boost™ and Ensure™; and

6) Miscellaneous Products–including eggs, processed foods such as soups, pre-prepared pasta sauces, pre-formed meals and the like.

The composition of the present invention may be incorporated directly and without further modification into the food, nutraceutical or beverage by techniques such as mixing, infusion, injection, blending, immersion, spraying and kneading. Alternatively, the composition may be applied directly onto a food or into a beverage by the consumer prior to ingestion. These are simple and economical modes of delivery.

There are some preferred techniques of enhancing solubility and dispersability which may work in concert with certain food manufacturing and/or processing methods. Solubility and dispersability of any phytosterol or phytostanol mixture may be enhanced

via the formation of emulsions and microemulsions which may readily be incorporated into margarines, butter, spreads, mayonnaise, dressings, yoghurt and the like. Patents covering the preparation of margarines and yellow spreads include: US Patent Serial Nos: 5,118,522; 5,536,523; 5,409,727; 5,346,716; 5,472,728; and 5,532,020, all of which are incorporated herein by reference.

EXAMPLES

Example 1: Cyclodextrin Complex

An aqueous ethanolic vehicle was prepared by mixing water and ethanol in the ratios of water (9 parts - 1 part) to ethanol (1 part - 9 parts) and the temperature was adjusted to 20-50°C. 2-dydroxypropyl-beta-cylcodextrin was dissolved in the mixture to give a concentration of 10-50% w/v, with stirring. A slight calculated excess of a phytosterol composition comprising beta-sitosterol, campesterol and stigmastanol was added in fine powder form to the mixture and the vessel sealed. The mixture was stirred for 2-48 hours under a maintained reaction temperature. The resultant mixture was filtered and the filtrate allowed to attain an appropriate temperature. The complex was then isolated by spraying drying at 39-90°C over an appropriate time cycle and yielded a free-flowing powder of small and regular particle size.

Example 2: Cyclodextrin Complex with Silicon Dioxide

The mixture was prepared in accordance with the protocol outlined in Example 1 up to and including the stage of filtration. In this example, 0.1-1% w/w of colloidal silicone dioxide (based on the calculated solids content of the filtrate) was added to the filtrate while stirring. The complex was then isolated by spray drying at 30-90°C over an appropriate time cycle and yielded a free-flowing powder of small and regular particles size.

Example 3: Complexation with Bile Salts

An aqueous ethanolic vehicle was prepared by mixing water and ethanol in the ratios of water (9 parts - 1 part) to ethanol (1 part - 9 parts) and the temperature was adjusted to 20-50°C. Calculated amounts of sodium cholate and sodium taurocholate were

dissolved to give a combined concentration of 30-60% w/v. the ratios of sodium cholate to sodium taurocholate were between 1 part - 9 parts to 9 parts - 1 part. A slight calculated excess of a phytosterol composition comprising beta-sitosterol, campesterol and stigmastanol was added in fine powder form, the vessel sealed, and the mixture gently stirred for 2-24 hours. The mixture was filtered and a calculated quantity of soybean lecithin (0.5-30% w/w based on the solids content of filtrate) was added with gentle stirring. Stirring was continued for a period up to 4 hours and the mixture allowed to attain a suitable temperature. The mixture was spray dried at 30-90°C over a suitable time cycle to yield an isolated solid complex as a free flowing powder of small and irregular particle size.

An enteric coating solution was prepared comprising a mixture of Eudragit™ L 100/ Eudragit ™ S 100 (enteric film formers) 6+1.2% w/w composite concentration, triethylcitrate (plasticiser) 0.6+1.12% w/w, talc (anti-tack agent) 3+0.6% w/w, water (vehicle) 5+1% w/w, isopropyl alcohol (vehicle) to 100% w/w. the powder, prepared as described above, was spray coated using equipment and methodology known in this field, to a percentage weight increase (based upon input weight of powder) sufficient to ensure an effective enteric barrier. The resultant coated powder product was then collected.

Example 4: Complexation with Bile Salts and Silicone Dioxide

The mixture was prepared in accordance with the protocol outlined in Example 3 up to and including the addition of soybean lecithin, stirring and cooling. In this example, 0.1-1% w/w of colloidal silicone dioxide (based on the calculated solids content of the filtrate) was added to the filtrate while stirring. The mixture was spray dried at 30-90°C over a suitable time cycle to yield an isolated solid complex as a free flowing powder of small and irregular particle size. The enteric coating solution was then prepared and applied as described in Example 3.

Example 5: Complexation with Bile Salts / Sub-coating with Water Soluble Film

The mixture was prepared in accordance with the protocol outlined in Example 3 up to and including the step of spray drying to form the free flowing powder. Prior to the

application of the enteric membrane, an initial sub-coating of a water-soluble film former, (here, hydroxypropylmethylcellulose formulated in a hydroalcoholic vehicle along with a plasticiser and ant-tack agent) was applied to the powder.

Example 6: Hydrotropic Complexation

Gentisic acid anhydride (10-40% w/v) was dissolved in appropriate volume of water, containing 5-20% v/v ethanol at 20-60°C. A calculate slight excess of a phytosterol composition comprising beta-sitosterol, campesterol and stigmastanol in fine powder form was added, the vessel sealed and the mixture stirred vigorously for 2-24 hours. The mixture was then filtered and the filtrate allowed to attain a suitable temperature. The mixture was spray dried at 30-90°C over a suitable time cycle and a solid hydrotropic complex isolated in free-flowing powder form, of small and regular particle size.

Example 7: Hydrotropic Complexation

The mixture was prepared in accordance with the protocol outlined in Example 6 up to and including the stage of filtration. In this example, 0.1-1% w/w of colloidal silicone dioxide (based on the calculated solids content of the filtrate) was added to the filtrate while stirring. The complex was then isolated by spray drying at 30-90°C over an appropriate time cycle and a solid hydrotropic complex isolated in free-flowing powder form, of small and regular particle size.

Unless otherwise stated for all examples hereinafter, FCP-3P2 Batch FM-P2-63 (composition: campestanol, 19.16%; sitostanol, 76.99%; campesterol, 0.13%; beta-sitosterol, 0.07%) was used in formulation work. Content uniformity data was referenced to the total phytostanol content of the batch, i.e. 96.15%.

Example 8: Solutions and Dispersions (Oil-based)

In order to determine the lipophilicity of FCP-3P2 the compound was evaluated in a selection of test systems. Solubility in fixed oils, the octanol / water partition coefficient and solubility in pH 5 aqueous buffer were chosen as relevant characteristics. Knowledge of these parameters would also act as guidance in future formulation efforts.

FCP-3P2 was represented by Batch FM-P2-48 (composition: campestanol, 20.03%; sitostanol, 75.12%, campesterol, 3.19%).

Solubility in Fixed Oils

This was determined by adding 500mg of test compound to 5mL of each oil and equilibrating by vortexing (VWR Multi-Tube Vortexer, setting 2) at 21 C for 16 hours, in 20mL closed glass scintillation vials. The vials were then centrifuged at 4000 rpm for 5 minutes and independently sampled for analysis by gas chromatography (GC-FID), using a cholestane internal standard. Results are presented in Table 1.

Table 1

Fixed Oil	FM-P2-48: TP
Canola	16.39
Corn	18.06
Olive	22.97
Peanut	19.08
Sesame	16.69
Key: TP = Total Phytosterols (campestanol + sitostanol + beta-sitosterol), concentration in mg/mL.	

The solubility of FM-P2-48 in selected fixed oils ranges from 16.39-22.97 mg/mL

Octanol / Water Partition Coefficient

The octanol / water partition coefficient was assessed by dissolving 5mg of test compound in 5mL of 1-octanol (oil phase), adding 15mL of pH 5.0 phosphate buffer and equilibrating by vortexing (VWR Multi-Tube Vortexer, setting 2) at 21 C for 30 seconds, followed by static storage at 21 C for 16 hours, in 20mL closed glass scintillation vials. It was observed that the two phases were completely transparent and no filtration step

was necessary prior to analysis. The vials were then independently sampled for analysis from the aqueous and octanol phases. Analysis was by GC-Mass Spectrometry.
Data for FCP-3P2 was not absolute, due to some technical difficulties with the analytical method, but again indicated that aqueous solubility was minimal and that the compound was essentially confined to the octanol phase. A previous experiment to assess the partition coefficient of this compound, using a related but modified experimental procedure and GC-FID detection also supported these observations.
Thus, it was not possible to determine formal partition coefficient values for the test compound, but it is clear that it is essentially lipophilic in nature.

Aqueous Solubility

This was evaluated by adding 15mg of test compound to 15mL of pH 5.0 phosphate buffer and equilibrating by vortexing (VWR Multi-Tube Vortexer, setting 2) at 21 C for 16 hours, in 20mL closed glass scintillation vials. Samples were withdrawn from the vials, filtered 0.2 microns and analysed by GC-FID, using a cholestane internal standard.
Results indicated that FCP-3P2 possesses negligible solubility in the aqueous buffer (below limit of quantitation of method, ie. less than 5ng/mL total phytosterols).

The above data indicates that FCP-3P2 is substantially lipophilic in character, having negligible solubility in simple aqueous media. In this respect, the data is consistent with comparable testing on steroids, which bear some significant structural similarities to the sterols and stanols.
The formulation of an oil-based solution of the active represents a feasible delivery system. If the quantity of FCP-3P2 exceeds it's solubility in the oily vehicle, a combination solution / dispersion will result. In this event, the active particle size distribution may be reduced, if desired, by homogenisation, e.g. using a high-shear device such as the Microfluidics Microfluidizer Model M-110Y or large-scale equivalent.

Example 9: Emulsions (Macroemulsions)

A 10% w/v solution of FCP-3P2 was prepared by adding 5.062g of material to 45.248g of soybean oil and heating to 63 C, to give a clear solution. 10mL of this solution was

taken and 0.748g of Span 60 [polyoxyethylene-(20)-sorbitan monostearate] dissolved in it. This constituted the oil phase. In this case, the surfactant has a Hydrophile-Lipophile Balance (HLB) value of 4.7 +/- 1.0.

Tween 40 [polyoxyethylene-(20)-sorbitan monopalmitate], 0.750g, was dissolved in 15mL of water, to provide the aqueous phase. Tween 40 has an HLB value of 15.6 +/- 1.0.

Both oil and aqueous phases were individually heated to 70 C, combined and vigorously mixed using a Polytron Model PCV II mixer, on the high speed setting, for 1 minute. The product was left to cool to ambient temperature.

This gave an oil in water emulsion, with an oil (dispersed) phase of 40% in an aqueous continuous phase, containing a dual surfactant system having an overall HLB of 10.0 +/- 1.0 and an active loading of ca 4% w/v (in the oil phase).

Analytical assessment of the emulsion included visual examination for phase separation over 5 days, optical microscopic evaluation of oil droplet size, pH measurement and FCP-3P2 content uniformity determination.

Phase Separation Assessment

15mL of emulsion was poured into a graduated centrifuge tube, which was subsequently sealed. Daily visual inspection over 5 days indicated no phase separation. Continued observation up to 25 days showed no separation of the two phases.

pH

The measured pH of the system was 6.63.

Oil Phase Droplet Size

This parameter was evaluated using an optical microscope equipped with a calibrated eyepiece. Sample preparation involved diluting 1 part of emulsion with 2 parts of water and examining a 15uL quantity on a microscope slide, under a cover slip, at 400x magnification. Phase contrast and cross-polarization conditions were utilised. The dispersed oil phase consisted of droplets ranging from ca 2.5-20 microns and no evidence of FCP-3P2 crystallisation was observed.

FCP-3P2 Content Uniformity Determination

This was assessed on 6 samples, removed from the bulk according to a pre-determined sample plan. Each sample (0.5mL) was extracted by vortexing for 10 minutes with dichloromethane (DCM, 5mL), followed by centrifugation at 4000 rpm for 2 minutes to separate the two phases. The analytical sample was withdrawn from the DCM layer and assayed by GC-FID, using a cholestane internal standard. Results are reported in Table 2.

Table 2

Sample #	TP
1	35.78
2	32.60
3	31.24
4	36.07
5	36.64
6	35.20
Mean Standard Deviation Theoretical Content (of test sample)	34.59 (93.2% of theoretical value) 2.16 37.11
Key: TP = Total Phytostanols (campestanol + sitostanol), concentration in mg/mL.	

Content uniformity is acceptable (34.59 +/- 2.16 mg/mL) and indicates satisfactory emulsion homogeneity. Recovery (93.2%) is a little low and this is probably due to pipetting errors.

Thus, this dosage delivery system has successfully enhanced both FCP-3P2 solubility and dispersibility.

Example 10: Self-Emulsifying Systems and Microemulsions

A self-emulsifying drug delivery system (SEDDS) is one that readily undergoes emulsification in aqueous media under low or modest shear (agitation) conditions. Elevated temperature is not necessarily required. This ideally translates to a spontaneous in vivo emulsification and subsequent dispersion following oral administration. Furthermore, it is desirable for the SEDDS to form a microemulsion on exposure to aqueous media, thereby affording an additional enhancement of

dispersibility and an increased surface area for absorption (smaller droplet size distribution than a macroemulsion).

A SEDDS may be utilised in a variety of ways. For example, it is suited to filling into a soft gelatin capsule (softgel), or other suitable dosage form, for oral administration, or it may be further processed into a microemulsion prior to administration.

One example of a SEDDS and it's subsequent compounding into a microemulsion is noted below.

Capmul MCM (a proprietary blend of medium chain glycerides), 5.30g, and Tween 80 [polyoxyethylene-(20)-sorbitan monooleate], 4.70g, were blended at ambient temperature, to form a transparent, completely-miscible, solution. FCP-3P2, 0.10g, was added and dissolved by sonicating for 10 minutes. A completely-transparent solution resulted. In this SEDDS formulation, Capmul MCM constitutes the oil phase and Tween 80 is a high-HLB (15.0 +/- 1.0) surfactant emulsifier.

Addition of 90mL of 0.9% w/v aqueous NaCl (ambient temperature, 5mL aliquots, gentle stirring-magnetic stir bar) affords a slightly opalescent microemulsion system.

Analytical assessment techniques were as per Macroemulsions.

Phase Separation Assessment

Daily visual assessment over 5 days indicated no phase separation.

pH

The measured pH of the system was 5.72.

Oil Phase Droplet Size

Dispersed phase droplets were below the level of visual detection (contrast with observation under Macroemulsions) and no evidence of FCP-3P2 crystallisation was noted.

FCP-3P2 Content Uniformity Determination

Results are reported in Table 3.

Table 3

Sample #	TP
1	0.303
2	0.372
3	0.417
4	0.400
5	0.404
6	0.415
Mean Standard Deviation Theoretical Content (of test sample)	0.385 (84.1% of theoretical value) 0.043 0.458
Key: TP = Total Phytostanols (campestanol+ sitostanol), concentration in mg/mL.	

Content uniformity is acceptable (0.385 +/- 0.043 mg/mL) and indicates satisfactory emulsion homogeneity. The mean recovery (84.1%) is a little low and is probably due to a combination of pipetting errors and the low concentration of active in the formulation.

Both of these delivery systems demonstrated improvements in FCP-3P2 solubility and dispersibility.

Example 11: Solid Dispersions

A cosolvent mixture, consisting of 125mL chloroform and 125mL ethanol, was prepared in a 500mL round-bottom flask.0.49g FCP-3P2 and 2.007g Benecel (a grade of hydroxypropylmethylcellulose) were added and the mixture was stirred at ambient temperature until a clear solution resulted. Solvent was removed by rotary evaporation under vacuum at 40 C and the resultant film was vacuum dried for a period at ambient temperature, following which the temperature was increased to 45 C and drying continued to achieve a total residual solvent level of less than 200 ppm (GC-headspace analysis). The dried film was cooled to ambient temperature and carefully scraped from the flask wall. This yielded a flaky powder, consisting of a hydrophilic cellulose matrix in which fine particles of FCP-3P2 were embedded.

Analytical assessment of product included: optical microscopic evaluation of FCP-3P2 particle size, differential scanning calorimetry (DSC), aqueous dispersibility testing, measurement of FCP-3P2 levels and X-ray diffraction (XRD) evaluation.

FCP-3P2 Particle Size

FCP-3P2 did not form a complete molecular dispersion in the Benecel matrix, following evaporation of the solvent vehicle. The complex film was visibly opaque and optical microscopic examination revealed rounded particulates, ranging from 20-200 microns in size, within the matrix.

Untreated FCP-3P2 generally exhibits a rod-like crystal habit and a significantly larger overall mean particle size and distribution. Modification of the crystallisation process (crystal habit and size) by polymeric and surfactant materials has been documented in the literature, so this observation is not unexpected.

Since the hydrophobic (lipophilic) FCP-3P2 in this preparation was of a markedly smaller overall particle size than the original material and is embedded in a hydrophilic water-soluble matrix, some improvement in aqueous dispersibility might reasonable be anticipated.

DSC Evaluation

DSC is widely employed to assess specific thermal properties of single materials and formulated systems. Examples would include determination of melting point and melting behaviour, identification of polymorphic forms, differentiation between amorphous and crystalline forms of a material and, in this case, evaluation of potential solid dispersion formation. Whilst characteristic melting endotherms for the individual components of a solid dispersion should be readily identifiable, conversion to a true solid dispersion would be expected to cause significant changes in their DSC thermograms. In the case of an active material, substantial modification or complete elimination of the specific melting endotherm(s) for that substance are commonly observed.

DSC scans were run on FCP-3P2, Benecel and the FCP-3P2/Benecel formulation, using a Dupont Model 910S Differential Scanning Calorimeter, calibrated against an indium standard, with helium gas purging. A scan rate of 10 C/minute, over a

temperature range of 20-200 C, was utilised. Sample sizes varied from 3.98-4.60mg and powders were run in crimped aluminium cups.

FCP-3P2 typically shows one major melting endotherm, with a peak value of approximately 143.3 C. Benecel exhibited no significant endo- or exo-therms over the test temperature range. The FCP-3P2/Benecel formulation showed a single endotherm at 142.6 C, corresponding to free FCP-3P2 and the area under the endotherm curve equated to the loading level of the FCP-3P2 in the matrix. Thus, we may say that a true molecular dispersion has not formed between these two substances.

Aqueous Dispersibility Testing

A model system was established in an attempt to ascertain whether specific formulation approaches could yield potential improvements in the dispersibility of FCP-3P2 in the gastric environment. For this purpose, a USP dissolution apparatus, equipped with paddles (Apparatus II) and domed vessels was employed. A paddle speed of 50 rpm and a dispersal medium comprising 300mL of 0.1N aqueous HCl at 37 C, were chosen as being reasonable test conditions. Stirring rate and medium volume were selected by experimentation to give efficient mixing without turbulence. A 60 minute overall assessment period was set.

Untreated FCP-3P2 is hydrophobic in nature. Material (50mg) added to the surface of the stirred test medium, did not wet and persisted as floating particles for up to 60 minutes, when the test was terminated.

The FCP-3P2/Benecel formulation sample was prepared by gently grinding in a mortar and passage through a 25 mesh sieve. Sieved material (100mg) was taken and added to the surface of the stirred test medium. Over the course of the 60 minute test period, material was observed to hydrate and commence dispersal into the medium. Whilst the dispersal process was not completed within this time, the test medium became noticeably opalescent. Optical microscopic examination of samples withdrawn from the bulk medium confirmed the presence of small particulates, as noted under 4.1.

Based upon this data, it would appear that the aqueous dispersibility of FCP-3P2 has been enhanced by this formulation approach. Wettability may be improved upon in future experiments, using a number of potential means. For example, addition of a suitable surfactant to the formulation, pre-suspension of the formulation in a quantity of

an appropriate water-miscible liquid vehicle, substitution of spray drying for rotary evaporation as a process for isolating a more uniform dried product, etc.

FCP-3P2 Content Uniformity Determination

FCP-3P2 was assayed by GC-FID, using a cholestane internal standard.
The theoretical recovery of FCP-3P2 from the formulation should be 19.6% w/w.
A random sample of powder yielded an assay result of 18.8% w/w (95.9% of theory).
This is an acceptable value, when allowances for sample processing and analytical variances are taken into account.

XRD Evaluation

The x-ray diffraction pattern of a substance can be used to evaluate it's internal structure and gives useful information as to whether a material is amorphous or crystalline in nature. The technique can also be used to demonstrate the influence of added substances on the pre-existing internal molecular arrangement of a particular material. As such, it constitutes a complimentary procedure to DSC investigations and was employed for this purpose in the current experimental work.
Material scans were conducted using a Rigaku Model D/MAX-2MB high resolution wide angle x-ray diffractometer. A sample size of approximately 2mL was required and material was scanned over a 2theta range of 5.0 to 40.0 degrees.
Scans were run on the individual components of the formulation, a physical mixture of the two components and the test solid dispersion.
FCP-3P2 showed a characteristic pattern that indicated a reasonable degree of crystallinity. Benecel also demonstrated a characteristic pattern, with little evidence of crystallinity. The physical mixture produced a composite pattern, containing elements of the individual components. FCP-3P2/Benecel solid dispersion showed a pattern which was quite similar to that of the physical mixture, but in addition appeared to indicate a reduced degree of FCP-3P2 crystallinity.
Thus, we may reasonably propose that the FCP-3P2 particulates in the Benecel matrix are present in a mixture of the amorphous and crystalline states, but that a true molecular dispersion has not formed. This observation is in agreement with the DSC results.

Application of this approach, or a modification thereof, should promote FCP-3P2 dispersibility in aqueous media.

Example 12: Suspensions

An example of an aqueous suspension is noted below.

Carbopol 971P (a proprietary grade of Carbomer 941 USNF), 0.51g, was added to water, 75mL, and mixed to create a smooth lump-free suspension.

Water, 10mL, glycerol, 5mL, and Tween 80 [polyoxyethylene-(20)- sorbitan monooleate], 0.014g, were blended together. FCP-3P2, 10g, was dispersed in this by vigorous mixing with a Polytron Model PCV II mixer (medium setting), to form a smooth, fully-wetted, dispersion concentrate.

The Carbopol and FCP-3P2 dispersions were mixed together and disodium edetate, 0.10g, was dissolved in the blend. 18% w/v aqueous KOH was carefully added to the dispersion, with mixing, until the pH stabilised at a value of 6.0. This step is required to partially neutralise the acidic Carbopol groups and establish a gel structure within the dispersion. Finally, the product was made to volume (100mL) with water and thoroughly mixed, to give a smooth suspension containing 100mg/mL FCP-3P2.

Analytical evaluation included: visual assessment of sedimentation, optical microscopic appearance, pH check and FCP-3P2 content uniformity determination. Test methods were as per Emulsions, unless otherwise stated.

Visual Assessment of Sedimentation.

Method as per Emulsions, Phase Separation Assessment.

Daily visual inspection over 5 days indicated that the suspension separated into two layers, from day 1 onwards. It readily re-suspended on gentle manual shaking following the day 5 observation point, i.e. it is a flocculated system. Such systems tend to maintain their re-suspendability characteristics more readily than formulations in which the dispersed phase is de-flocculated and ultimately tends to agglomeration and caking.

Optical Microscopic Appearance

This characteristic was evaluated using an optical microscope equipped with a

calibrated eyepiece. The suspension was examined in the undiluted form, by placing a suitable quantity on a microscope slide and fitting a cover slip, at 400x magnification and ranged from approximately 2.5 to 25 microns in particle size (major axis).

pH

The measured pH of the system was 6.0 (initial) and 5.9 at the 5 day test point of the sedimentation test.

FCP-3P2 Content Uniformity Determination

Results are presented in Table 4.

Table 4

Sample #	TP
1	0.730
2	0.792
3	0.890
4	0.790
5	0.750
6	0.761
Mean Standard Deviation Theoretical Content (of test sample)	0.785 (85.8% of theoretical value) 0.056 0.9153
Key: TP = Total Phytostanols (campestanol+ sitostanol), concentration in mg/mL.	

Content uniformity is acceptable (0.785 +/- 0.056 mg/mL) and indicates satisfactory homogeneity of the dispersed active. The mean recovery (85.8%) is a little low and this may be due to pipetting errors and/or recovery problems.

Thus, this approach has enhanced FCP-3P2 dispersibility in aqueous systems.

Example 13: Hydrated Lipid Systems

A liposomal dispersion was evaluated as an example of a hydrated lipid system.

A solution was prepared containing the following materials: phospholipids-dimyristoylphosphatidylcholine, 2.832g, dimyristoylphosphatidylglycerol, 1.392g; FCP-3P2, 0.782g; dichloromethane, to 50mL. Sonication of the mixture for 30 minutes at 40 C

yielded a slightly opalescent solution, which was subsequently clarified by passage through a 0.5 micron filter.

The solution was transferred to a 125mL round bottom flask and solvent removed by rotary evaporation under vacuum at 30-40 C, 35 rpm flask speed, to give a coherent thin film covering a substantial portion of the interior surface of the flask. Continued ambient temperature vacuum drying of the resultant film yielded a product with a residual solvent level of less than 200ppm dichloromethane (GC-headspace analysis).

50mL of a 5% w/v aqueous glucose solution was added to the flask and the thin film was hydrated by rotating for 1 hour at 40 C and 60 rpm flask speed (no vacuum). A temperature of 40 C is well above the critical temperature of the phospholipids (Tc = 23 C) and ensures that the membranes of the liposomal vesicles remain in a suitably fluid state to facilitate further processing. Hydration was completed by gentle stirring (magnetic stir bar) for 1 hour, at a temperature above the Tc value. This created a dispersion of large multilamellar vesicles (LMVs). The particle size and lamellarity of the LMVs was reduced by passing 250 uL quantities of dispersion (at 40 C) through an Avanti Mini Extruder, fitted with a 0.08 micron filter, for a total of 11 passes, to give a dispersion of small unilamellar vesicles (SUVs).
Analytical assessment of the formulation included: liposomal particle size evaluation, pH measurement and FCP-3P2 content uniformity determination.
Testing methodologies were as per Emulsions, unless otherwise stated.

Liposomal Particle Size Assessment

The SUV dispersion was examined under 400x magnification and in the phase contrast mode. A uniform dispersion of discrete liposomes having diameters of less than 1 micron was observed.

pH

The measured pH of the system was 6.45.

FCP-3P2 Content Uniformity Determination

Initial attempts at performing FCP-3P2 content uniformity testing of the liposomal dispersion did not meet with success. Solubilisation of the liposomes with surfactant agents (0.5% Tween 80 and 0.5% sodium lauryl sulfate, used individually) appeared to lyse the liposomes, but the existing analytical procedure failed to extract FCP-3P2 from the solubilised solutions. This may indicate that the active is tightly associated with one or more of the phospholipids and further studies will be required to investigate the matter.

This formulation approach has enhanced both the solubility and dispersibility of the active in aqueous media.

Example 14: Cyclodextrin Complexation

This formulation approach was investigated by application of the paste formation method.

2-Hydroxypropyl-beta-cyclodextrin (2-HPBC), 7.30g, and FCP-3P2, 0.88g, were thoroughly blended together in a glass vessel. 2 mL of a 30% v/v aqueous ethanol solution was added slowly, with mixing, to form a smooth thick paste and the vessel was loosely closed. The product was placed at 115 C for 2 days, to facilitate inclusion complex formation and subsequent evaporation of residual solvents. This yielded a dry friable mass, which was removed to ambient temperature and the vessel tightly sealed.

Analytical evaluation of the product included: DSC assessment, XRD evaluation, aqueous dispersibility testing and FCP-3P2 content uniformity determination. Testing methods were as per Solid Dispersions, unless otherwise stated.

DSC Evaluation

DSC scans were run on the individual components and the test formulation. Sample sizes varied from 3.74-4.67mg. The test temperature range was 20-350 C.

2-HPBC showed no significant thermal events over the temperature range 20-300 C, but a modest progressive rise in the thermogram was noted, followed by a sharp melting from approximately 305 C onwards. The FCP-3P2/2-HPBC formulation showed one broad exotherm with a peak at 214.6 C and no melting endotherm for the active (a peak at 143.3 C is typical for pure FCP-3P2). A complex melting

endotherm was noted at approximately 245 C, probably due to a lowered melting point for the 2-HPBC.

Thus, it is clear that an inclusion complex has formed between the two substances.

XRD Evaluation

Scans were run on the individual components of the formulation and the complex. 2-HPBC showed no evidence of crystallinity and appeared to be amorphous in nature. The characteristic FCP-3P2 peaks were absent in the scan of the complex, which, again, seemed to be amorphous in structure.

This data supports the DSC observations and indicates the formation of an inclusion complex between FCP-3P2 and 2-HPBC.

Aqueous Dispersibility Testing

The FCP-3P2/2-HPB sample was prepared by gentle grinding in a mortar and easily reduced to a fine powder.

At 5 minutes, most of the material had wetted and entered solution as a fine suspension.

By 60 minutes, all material was uniformly suspended in solution, with only a trace of solids remaining at the surface.

Complexation with 2-HPBC has facilitated dispersibility of FCP-3P2 in aqueous media.

7.4. FCP-3P2 Content Uniformity Determination

Results are reported in Table 5.

Table 5

Sample #	TP
1	0.423
2	0.343
3	0.403
4	0.371
5	0.391
6	0.451
Mean Standard Deviation Theoretical Content (of test sample)	0.397 (34.6% of theoretical value) 0.038 1.146
Key: TP = Total Phytostanols (campestanol + sitostanol), concentration in mg/mL.	

Reviewing Table 5 data, the mean recovery is very low, at 34.6% of theoretical. Since the sample preparation procedure involves extraction into DCM prior to analysis, two potential explanations suggest themselves. FCP-3P2 is adequately soluble in DCM, whilst 2-HPBC has a negligible solubility in this solvent. Under these circumstances, it is possible that the data recorded represents free FCP-3P2 that has not formed an inclusion complex with 2-HPBC. By inference, 64.4% of active should be present as the complex. An alternative possibility is that the extraction process has only recovered a percentage of FCP-3P2 from the inclusion complex. DCM is a lipophilic solvent which possesses a reasonable degree of polarity. Therefore, one would expect it to show some affinity for included FCP-3P2. The DSC and XRD data clearly indicate inclusion complex formation between the two substances and the former shows no melting endotherm for uncomplexed FCP-3P2. Therefore, it is felt that the latter explanation is the most likely to be correct. Considered in this light, the content uniformity data tends to suggest a homogeneous product with respect to FCP-3P2 distribution.

This formulation approach has enhanced the dispersibility of the active in aqueous media.

Example 15 Complexation with Bile Salts

Sodium deoxycholate (SDC) was chosen as a typical example of a human bile salt.

A cosolvent mixture, consisting of 100mL ethanol and 50mL DCM, was added to a 500mL round-bottom flask. FCP-3P2, 1.027g, and SDC, 4.008g, were added and the flask manually swirled, to give a clear solution at ambient temperature. Solvent was removed by rotary evaporation under vacuum at 40-50 C, to yield a white powder mass. Further vacuum drying at ambient temperature reduced residual solvent to workable levels (DCM less than 200 ppm, ethanol greater than 200 ppm- limit testing employed; GC-headspace analysis). The product was free of residual solvent odour.

Analytical investigation of product included: DSC assessment, XRD evaluation, aqueous dispersibility testing and FCP-3P2 content uniformity determination. Testing methods were as per Solid Dispersions, unless otherwise stated.

DSC Evaluation

DSC scans were run on the individual components and the test formulation. Sample sizes varied from 3.54-3.59mg.

SDC showed no significant thermal event over the test temperature range.

The thermogram for the test formulation showed two consecutive melting endotherms, peaking at 136.3 C and 141.8 C, separated by a small exothermic peak. FCP-3P2 typically has a melting endotherm peaking at 143.3 C. Thus, these thermal events are probably due to the active. One possible explanation is that FCP-3P2 may exist in a metastable state in the formulation, initially melting at 136.3 C, recrystallising to the stable form and then re-melting at 141.8 C. It was noted that the area under the 141.8 C endotherm corresponded to the quantity of active contained in the formulation. This indicates that FCP-3P2 has not formed a molecular dispersion with SDC.

XRD Evaluation

Scans were run on the individual components and the test formulation.

SDC showed a pattern that is indicative of a non-crystalline material.

The test formulation scan was essentially a composite of the patterns for the two components and the degree of crystallinity of the FCP-3P2 has been little affected by it's combination with SDC. These observations support the DSC data.

Aqueous Dispersibility Testing

The test formulation presented as a fine powder and was used as received.

In this case, pH 5 phosphate buffer was used as the test medium, since the bile acid is present as it's sodium salt, which possesses a significantly greater aqueous solubility than the parent acid. Contact with 0.1N HCl, the medium initially chosen for dispersibility testing, would cause conversion to the acid form and substantially inhibit dispersion of the complex. This consideration necessitates the design of a dosage form which releases it's contents upon reaching the upper regions of the small intestine and is protected from exposure to the acidic gastric fluid, i.e. an enteric coated system. Since bile salts are known to cause gastric irritation and emesis upon oral administration, these would be further reasons to prevent

premature release in the stomach.

At 5 minutes, the test formulation had substantially wetted and appeared as a fine particulate dispersion in the test medium, with a small proportion of material floating at the surface. By 60 minutes, only a trace of material was present at the surface, the majority being present as a uniform particulate suspension.

This formulation has demonstrated an enhanced dispersibility in aqueous media (pH 5.0 and above).

FCP-3P2 Content Uniformity Determination.

Results are reported in Table 6.

Table 6

Sample #	TP
1	1.575
2	1.759
3	1.938
4	1.750
5	1.586
6	1.507
Mean Standard Deviation Theoretical content (of test sample)	1.686 (91.2% of theoretical value) 0.159 1.848
Key: TP = Total Phytostanols (campestanol + sitostanol), concentration in mg/mL.	

Content uniformity is acceptable(1.686 +/- 0.159 mg/mL) and indicates satisfactory FCP-3P2 homogeneity. The mean recovery (91.2%) is a little low and this is probably due to pipetting errors.

This formulation approach has improved FCP-3P2 dispersibility and wettability in aqueous media. Since the bile salts perform a solubilising function in vivo (they are surfactants), it is also possible that oral bioavailability will be enhanced.

Example 16: Hydrotropic Complexation

Sodium gentisate (SG) was selected as a model hydrotrope.

FCP-3P2, 2.066g, and SG, 4.242g, were added to 200mL of ethanol and the mixture was sonicated at 60 C to achieve complete dissolution. The resultant solution was transferred to a 250mL round bottom flask and solvent removed by vacuum rotary drying at 60 C, to give a damp mass. Further vacuum drying at ambient temperature yielded a dry powder mass.

Analytical testing included: DCS assessment, XRD evaluation, aqueous dispersibility testing and FCP-3P2 content uniformity determination. Test methods were as per Solid Dispersions, unless otherwise stated.

DSC Evaluation

DSC scans were run on the individual components and the test formulation.

The SG thermogram showed two melting endotherms, peaking at 89.7 C and 169.6 C.

The test formulation thermogram showed a similar pattern to that for the SDC (Bile Salt) complex, consisting of a minor melting endotherm peaking at approximately 135 C, followed by a small exotherm, leading to a sharp endotherm peaking at 141.5 C (FCP-3P2 typically shows a sharp melting endotherm at 143.3 C). A similar explanation is proposed for this behaviour (refer to section on Bile Salts Complexation). The two melting endotherms for SG were eliminated, indicating a potential modification of it's crystalline structure).

It would seem from this data that FCP-3P2 has not formed a molecular dispersion in SG.

XRD Evaluation

Determinations were made on the individual components and the test formulation.

FCP-3P2 and SG both demonstrate well-defined peak patterns, suggesting a significant crystalline component to their structures.

The test formulation showed little evidence of crystallinity, indicating that some definite physical interaction has occurred between the two substances. This observation is consistent with the DSC evaluation data.

Aqueous Dispersibility Testing

Again, pH 5.0 phosphate buffer was substituted for 0.1N HCl, since the sodium salt of gentisic acid was present in the formulation.

Material initially floats on the surface of the test medium, with some particulates dispersing into the bulk liquid. A visible film forms at the surface, indicating some dissolution of the SG carrier. At 60 minutes, there was a slight increase in the level of dispersed particles, but the majority of the material remained at the surface and the SG film persisted.

This approach has not been successful in improving either the solubility or dispersibility of FCP-3P2 in aqueous media.

FCP-3P2 Content Uniformity Determination

Results are reported in Table 7.

Table 7

Sample #	TP
1	1.622
2	1.922
3	1.896
4	1.740
5	1.811
6	1.882
Mean Standard Deviation Theoretical Content (of test sample)	1.812 (121.1% of theoretical value) 0.114 1.496
Key: TP = Total Phytostanols (campestanol + sitostanol), concentration is in mg/mL.	

The apparent mean recovery (121.1%) is high and this will require further investigation. Matrix interference effects are one possible explanation.

Content uniformity is acceptable (1.812 +/- 0.114mg/mL) when taken on the basis of relative comparison and indicates satisfactory homogeneity of the dispersed active.

This formulation approach has not significantly enhanced the solubility or dispersibility of FCP-3P2 in aqueous media (pH 5.0 and above).

Example 17: Yogurt

Phytrol® which consists of campesterol, campestanol, β-sitosterol and sitostanol was

mixed with nonfat milk powder in the ratio of 1:7 to 1:8. About 6 L of milk mix was prepared from whole milk, skimmed milk and phytrol containing milk powder. Milk was standardized to 0.75 – 1% fat, 12 – 13% solids and 0.5-1% phytrol using the Pearsons Square method (Hyde, K.A. and Rothwell, J., 1973, In Ice Cream, Churchill Livingstone Ltd., London, U.K.). Milk mix was permitted to remain at room temperature for 30 minutes to re-hydrate powder milk and than it was homogenized using a high sheer batch mixer (Ultra-Turrax T50 equipped with the dispersing element S50N, IKA Works Inc., Wilmington, NC, USA). Other devices such as a single-stage homogeniser, a two-stage homogeniser or a high-pressure microfluidizer may alternatively be used for homogenization of the milk mix. Next, milk mix was pasteurized at 69oC (156oF) for 30 minutes (batch/vat), cooled to 44oC and hold at this temperature for up to 15 minutes.

About 3% by weight of active yogurt culture containing Lactobacillus bulgaricus and *Streptococcus thermophilus* in the ratio 1:1 were carefully introduced into warm milk mix. After gentle mixing, the inoculated milk was distributed into 125 g-containers filling to near top. The containers were thermally sealed with aluminum leads and placed in incubator (44°C) equipped with good uniform air circulator and temperature controller. Filled containers were permitted to remain at 44°C for 3-5 hours, until a firm, smooth gel was formed. During incubation, pH was monitored periodically. When pH reached about 4.5, yogurt was withdrawn from the incubator, chilled quickly and stored at 4°C.

Example 18: Bread

Breads containing 0.6% and 1.2% of Phytrol were prepared using bread maker (Black & Decker, Model # B2005). Phytrol® consisted of campesterol campestanol, β-sitosterol and sitostanol was mixed with multipurpose flour (1% and 2%, w/w) using Hobart mixer (Model N50). Alternatively, Phytrol was mixed with milk using a high-pressure microfluidizer. Subsequently, all other ingredients were mixed in proportions indicated below.

Ingredients	0.6% Phytrol (g)	1.2% Phytrol (g)
Milk	334.00	334.00
Salt	7.50	7.50
Sugar	7.10	7.10
Crisco	12.00	12.00
Flour	535.00	535.00
Phytrol	5.42	10.84
Yeast	2.80	2.80

Ingredients were combined in the baking pan of bread maker. Preparation of dough and baking was conducting according the manufacturing instructions.

Example 19: Cereal Bar

Cereal bars of total weight 20g, and 40g that contained 3%, and 1.5% of Phytrol, respectively, were prepared. Phytrol® consisted of campesterol, campestanol, β-sitosterol and sitostanol was dissolved in partially hydrogenated vegetable oil in elevated temperature (40-80oC). The oil/Phytrol blend was cooled to 30oC and emulsified using a high sheer batch mixer (Ultra-Turrax T50 equipped with the dispersing element S50N, IKA Works Inc., Wilmington, NC, USA). Subsequently, two oil blends (9.4% and 18.8% of Phytrol) were further emulsified using a high-pressure microfluidizer at 20,000 PSI.

Cereal bars were produced by combining binder (40%), water (5%) and edible particles (55%). Below two typical examples of binder used for making a cereal bar.

Sucrose containing binder

Phytrol (9.4% or 18.8%) containing oil	40%
Sucrose	22%
Water	28%
Sodium Caseinate	5%
Lecithin	2%
Glycerin	3%

Glucose containing binder

Phytrol (9.4% or 18.8%) containing oil	40%
Glucose syrup	50%
Sodium Caseinate	5%
Lecithin	2%
Glycerin	3%

Sucrose in water /glucose syrup was heated to 100oC while Phytrol containing fat was liquefied at 40-80oC. Hot sugar solution was placed in the bowl (Hobart mixer, Model N50) and fat was added followed by adding all remaining binder ingredients. All ingredients were thoroughly and vigorously mixed. After cooling down to 40oC, edible particles are added while thorough, non-vigorous mixing was carried out. Following edible particles were typically incorporated into the cereal bars.

Edible particles

Rolled oats	20-40%
Crisped rice	10-20%
Puffed barley	10-20%
Dried apple dices	10-20%
Shredded coconut	5-10%
Raisins	5-10%
Various nuts	5-10%

After mixing was completed, mixed material was placed in the forming mold and pressed with a roller. After removal from the mold, it was cut into ready to eat various sizes cereal bars.

Example 20: Spread

Light margarine (60% fat) containing 6% of Phytrol was produced in batches of 5-10kg. Phytrol® consisted of campesterol, campestanol, β-sitosterol and sitostanol was combined with other fat ingredients and heated to 80-85oC until Phytrol was fully dissolved. Clear fat solution was placed in the feeding tank (20L), cooled to 40-45 oC and stirred using (Ultra-Turrax T50 equipped with the dispersing element S50N, IKA Works Inc., Wilmington, NC, USA). Next, the water fraction (40%) was added and temperature was adjusted to 60 oC. The blend was submitted into a votator and processed at 8-10oC. The composition of margarine is describe above.

Ingredient	Wt%
Water Phase	
Water	39.0
Salt	1.0
Potassium sorbate	0.001
Oil Phase	
Soybean oil	38.025
Palm kernel oil	15.0
Phytrol	6.0
Mono/diglycerides	0.6
Lecithin	0.15
Flavor	0.075
Beta-carotene	0.15

Example 21: Chocolate

Milk chocolate containing 6% of Phytrol was produced in batches of 20-50kg. Phytrol® consisted of campesterol, campestanol, β-sitosterol and sitostanol was mixed with soybean oil using a high sheer batch mixer (Ultra-Turrax T50 equipped with the dispersing element S50N, IKA Works Inc., Wilmington, NC, USA). The blend (20% Phytrol) was subsequently emulsified using a high-pressure microfluidizer at 20,000 PSI. Chocolate was composed of an outer shell (42 wt%, no Phytrol) and a center (69%, Phytrol). Chocolate outer shell was made by mixing sugar (45%), whole milk powder (20%), cocoa butter (23%), cocoa mass (12%), soy lecithin (0.3%) and pure vanilla (0.1%) in a heating tank. All ingredients were melted, tempered and deposited into molds. Center was prepare my mixing sugar, cocoa butter, whole milk powder, cocoa mass, soy lecithin and pure vanilla in the proportions as for outer shell. The mix was melted and tempered. Consequently, Phytrol/soybean oil blend was mixed with chocolate in the 1:1 ratio and deposited into molds previously filled with chocolate without Phytrol. Chocolate pieces were than cooled, wrapped and packed into the boxes. Using the molding system, 10-12 g chocolate pieces were produced.

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We Claim:

1. A composition suitable for incorporation into foods, beverages, pharmaceuticals, nutraceuticals and the like which comprises one or more phytosterols, phytostanols or mixtures of both, treated to enhance the solubility and dispersability thereof.

2. The composition of claim 1 wherein the phytosterols are selected from the group consisting of sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized, isomeric forms and derivatives thereof.

3. The composition of claim 1 wherein the phytosterols, phytostanols or mixtures of both are treated to enhance solubility via the formation of either an emulsion or microemulsion.

4. The composition of claim 1 wherein the phytosterols, phytostanols or mixtures of both are treated to enhance solubility by a technique selected from the group consisting of: formation of solid dispersions, formation of suspensions, formation of hydrated lipid systems, or includion complexations with cyclodextrins, hydrotopes and bile salts.

5. A pharmaceutical formulation for lowering serum cholesterol in animals comprising one or more phytosterols, phytostanols or mixtures of both, treated to enhance the solubility and dispersability thereof.

6. A food comprising one or more phytosterols, phytostanols or mixtures of both, treated to enhance the solubility and dispersability thereof.

7. A beverage comprising one or more phytosterols, phytostanols or mixtures of both, treated to enhance the solubility and dispersability thereof.

8. The use of a composition comprising one or more phytosterols, phytostanols or mixtures of both, treated to enhance the solubility and dispersability thereof, to lower serum cholesterol in animals.

9. A method of enhancing the solubility and dispersability of one or more phytosterols, phytostanols or mixtures of both which comprises incorporating one or more phytosterols, phytostanols or mixtures of both into one of the systems selected from the group consisting of: an emulsion, a microemulsion, a solid dispersion, a suspension, a hydrated lipid system, and an inclusion complex with cycolodextrins, hydrotopes and bile salts.

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/CA 99/00512

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23L1/03 A61K31/575 A23L2/52 A23L1/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 839 458 A (RIKEN VITAMIN CO., LTD.) 6 May 1998 (1998-05-06) page 3, line 3-8; claims 1,4,6,7 ---	1-4,6,9
A,P	WO 99 21534 A (MERCK PATENT GMBH) 6 May 1999 (1999-05-06) page 4, line 6-23 ===	1,4,9
A	US 5 244 887 A (STRAUB CARL D.) 14 September 1993 (1993-09-14) the whole document ---	1,4,9
A	WO 96 10033 A (THE UNIVERSITY OF BRITISH COLUMBIA) 4 April 1996 (1996-04-04) cited in the application ---	
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
14 October 1999	05/11/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Caturla Vicente, V

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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